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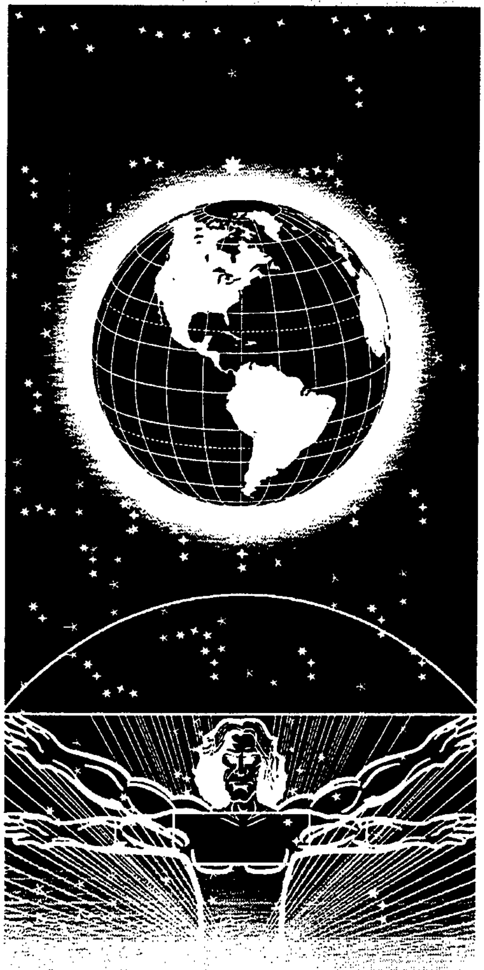

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

CARDIOVASCULAR RESPONSE IN MALE SPRAGUE-DAWLEY RATS MEASURED BY RADIOTELEMETRIC IMPLANTS AND TAILCUFF SPHYGMOMANOMETRY

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NOTICES

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Air Force Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

STEPHEN R. CHANNEL, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Armstrong Laboratory

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Tailcuff sphygmomanometry (Tailcuff) was compared to radiotelemetric implants (Implants) for monitoring blood pressure and heart rate in male Sprague-Dawley rats. Tailcuff has the intrinsic advantage of being noninvasive. However, Tailcuff requires at least several days (or more) of animal training to eliminate pulse artifacts generated by stress or gross body movements from close containment within unfamiliar surroundings. There is no training involved with implantation. Although several days are needed to insure recovery of the animal from surgery, no long-term, overt physiological stress was detected in animals subjected to implantation in terms of weight gain, liver to whole body ratios, gross changes in liver histology, or changes in blood pressure or heart rate (as measured by Tailcuff). With one exception, correlations of tailcuff readings to each other were substantially lower than correlations of implant readings to each other, indicating that the implant readings were more internally consistent. Correlations of blood pressure and heart rate parameters between tailcuff and implant measurements were either modest or not significant. The implantation method has one important advantage over Tailcuff: animals can be monitored continuously day and night without the need for interfering with animal activity.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, OH. This document serves as an interim report on monitoring cardiovascular response of Sprague-Dawley rats in preparation for future studies involving exposure of rats to nitrated explosives or propellants. The research described in this report began in April 1994 and was completed in February 1995 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. A07). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was sponsored by the U.S. Army under the direction of LTC Daniel J. Caldwell, USAMRD/WRAIR, USA.

The animals used in this study were handled in acccordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on the Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHS, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors wish to thank Col. J. Cooper and Ms. S. Young for their assistance in the implantation of the radiotelemeters, Ms. Young for necropsy, Maj. G. Marit for pathological evaluations, Mr. W. Malcomb for assistance in the construction of the ambient temperature chamber, and Mr. D. Fell for DTIC and other library database searches. Mr. D. Pollard's suggestion for using styrofoam to warm up the rat tails is also gratefully acknowledged.

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ABBREVIATIONS

C	Celsius
DIA	Diastolic blood pressure
g	Gram
HR	Heart rate
kg	Kilogram
kHz	Kilohertz
MBP	Mean blood pressure
mg	Milligram
min	Minute
ml	Milliliter
mmHg	Millimeters of mercury
N	Number
p	Probability
SEM	Standard error of the mean
SYS	Systolic blood pressure

SECTION 1

INTRODUCTION

Traditional methods for monitoring the effects of pharmacologically active chemicals on the cardiovasculature of animals involve direct cannulation of major arteries and injection of drugs into the vessels of anesthesized animals. The direct injection of drugs into anesthetized animals is not a suitable model for determining the effects of inhalation, ingestion, or dermal exposure of conscious individuals to pharmacologically active agents. Furthermore, direct cannulation requires invasive procedures for placement of cannulas, maintenance of patency in the externalized portion, and a physical connection between the exteriorized portion and a blood pressure/heart rate monitor. It has been reported that long-term, chronic in-dwelling arterial catheters adversely affect food consumption and weight gain in rats (O'Neill and Kaufman,1990).

A noninvasive alternative to direct cannulation of animals is tailcuff monitoring. A variety of studies have been done to validate this procedure against direct cannulation (Lucas, 1971; Andrews and Jones, 1978; Palbol and Henningsen, 1979; Yamakoshi et al., 1979; Borg and Viberg, 1980; Bunag and Butterfield, 1982; Borkowski and Quinn, 1983; Bunag, 1983; Wen et al, 1988; Ferrari et al., 1990; Kuwahara et al., 1991; O'Neill and Kaufman, 1990; Ikeda et al., 1991; Spanos et al., 1991). Advantages over direct cannulation include no surgical trauma and the ability to administer doses by multiple routes. A disadvantage to this method is the need to restrain the animal to minimize movements that interefere with readings. Therefore, animals must either be trained to remain calm within restraints or anesthetized. In rodents, additional problems arise because of their ability to use the tail artery as a thermoregulator. Body temperatures must be elevated to induce a tail pulse. However, excessive heating can cause artifactual increases in blood pressure (Wen et al., 1988; Kuwahara et al., 1991). Under ideal conditions Tailcuff appears to correlate well with direct cannulation, although readings are generally 5 mmHg lower than those obtained by the direct cannulation method.

A third and more recent approach for measuring blood pressure is cannulation of a major artery with an implantable transmitter that is sewn in place inside a body cavity (Brockway et al., 1991). Typically, the lower abdominal aorta is cannulated with the transmitter body sewn to the

peritoneal cavity wall. The technique has been validated against long-term, direct, tethered cannulation (Guiol et al., 1992; DePasquale et al., 1994). The animal is allowed to recover and then placed in its cage near a receiving unit that detects radio signals emitted by the implanted telemetry device. The receiver converts the radio waves into electronic signals that are sent to a computer and recorded as blood pressure, heart rate, temperature, electrocardiograms, or combinations thereof. The principal advantage of this technique over conventional cannulation is the elimination of an exteriorized cannula that may have problems with patency and sterility. The elimination of the requirement that an animal be tethered to a blood pressure monitor removes a potential source of stress and concommittent undesirable rises in baseline blood pressure. Furthermore, the animals can be monitored 24 hours a day without continuous supervision. Disadvantages include the requirement for relatively expensive transmitters which must be returned to the manufacturer for battery replacement and refurbishment, electrical interference of transmitter signals from other instruments, and electronic "drift" of transmitters over time.

SECTION 2

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, approximately 200-250 grams at receipt and 467-501g at treatment, were purchased from Charles River Breeding Laboratories, Raleigh, NC, and provided with food (Purina Rodent Chow #5008 (Ralston, Purina, St. Louis, MO) and water *ad libitum*. Animals were quarantined for a two-week period during which standard quality control procedures were performed. Rats were single housed in clear, plastic shoe-box type cages with wood-chip bedding (Bettachip®, Northeastern Products Corp., Warrensburg, NH). All rats were identified by tail tattoo.

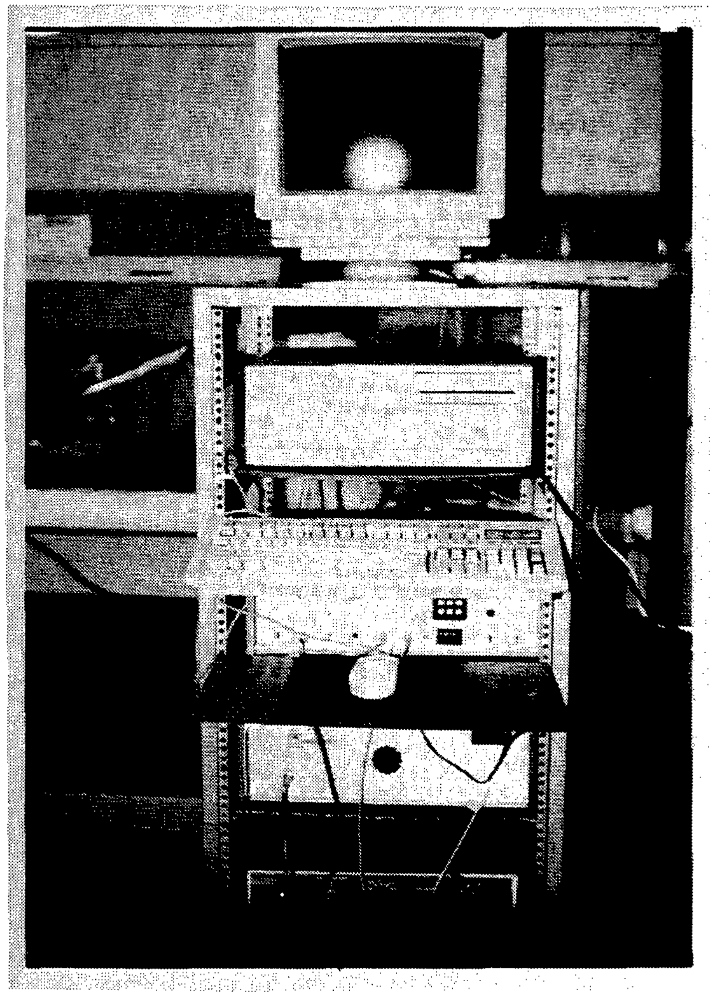
Tailcuff Monitoring

Equipment

A Model 179 blood pressure analyzer, Model 65-12 manual scanner, Model 27 cuff pump, Model 31 adapter board firmware (V1.00, 1994), and Blood Pressure Monitor Data View Program (Version 2.19, 1994) were purchased from IITC, Inc., Woodland Hills, CA. A GTSI 486 computer with 8 megabytes of RAM, 340 megabyte hard drive, math coprocessor, R232 interface, and DOS software (Version 6.21) was installed with the IITC firmware board. Included with the computer was a CTX color monitor and a mouse. The computer was connected to the blood pressure analyzer, scanner, and pump (see photograph in Figure 1a and diagram in Figure 1b). The equipment was calibrated daily prior to each use according to the manufacturer's instructions with a calibrated pressure gauge.

Animal Training

Animals were acclimatized to walk-in, plexiglas, tubular restraints (developed in-house) in preparation for tailcuff monitoring (see photograph in Figure 2a and diagram in Figure 2b).
This involved placing the tubular insert into the restraint tube when required for animals that are slightly smaller than the restraint tube to prevent them from turning around. The anterior restraint cover slides onto the front of the restraint tube and is tightened in place with a hand nut.



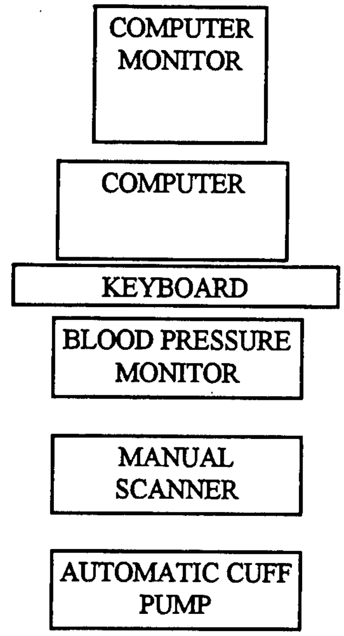


Figure 1b. (diagram)

Figure 1a. (photo) **Tailcuff Hardware**

The animal is allowed to walk into the rear of the restraint tube. If the animal is apprehensive, the tube may be covered with a paper bag to shut out light and encourage entry. Once the animal is inside, the posterior restraint cover is placed over the rear entrance, the tail is placed into the tail opening and the cover is snapped into place.

The entire tail should extend out as far as possible from the restraint tube and the anterior cover should be adjusted further into the restraint tube to minimize animal movement and slippage of the tail back into the tube. The pressure module is fitted as close to the base of the tail as possible and bolted onto the posterior restraint cover with hand nuts. The final assembly is shown in the photograph in Figure 3a and diagram in Figure 3b. The tubes and accessories were cleaned with only warm water (mild soap may also be used) because harsh detergents can have an effect on rats similar to amphetamines.

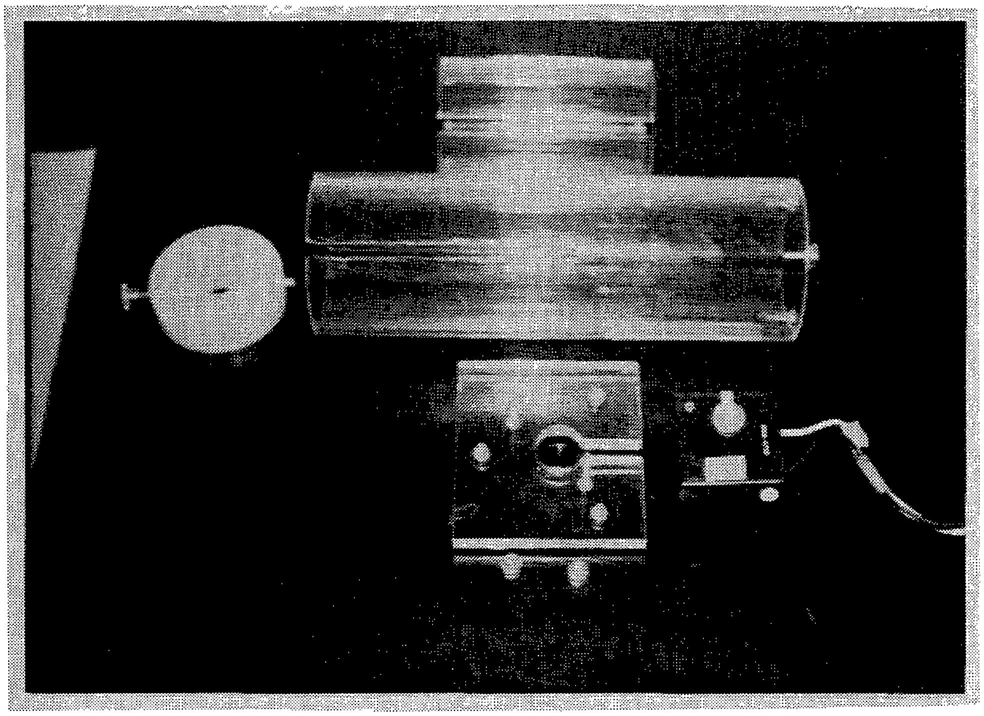


Figure 2a. Rat Restraint (photo)

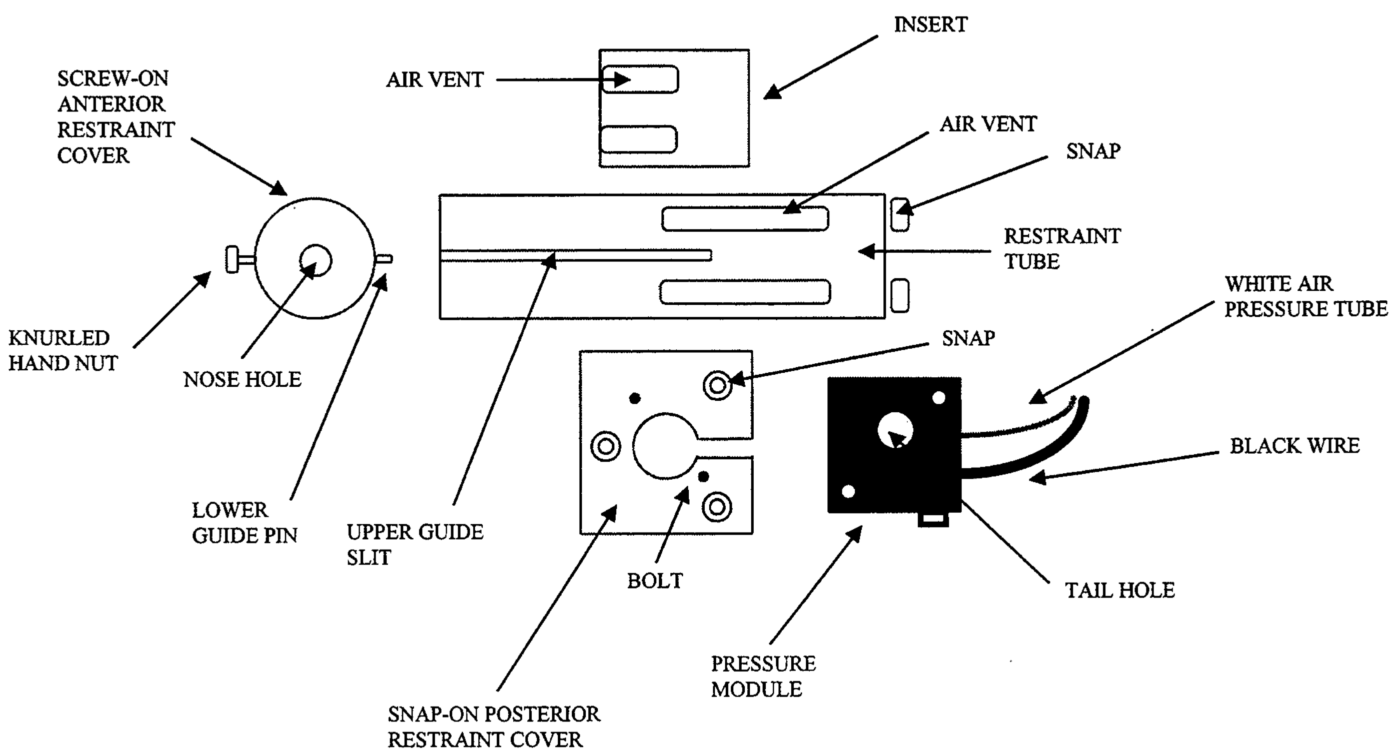


Figure 2b. Rat Restraint (diagram of exploded view)

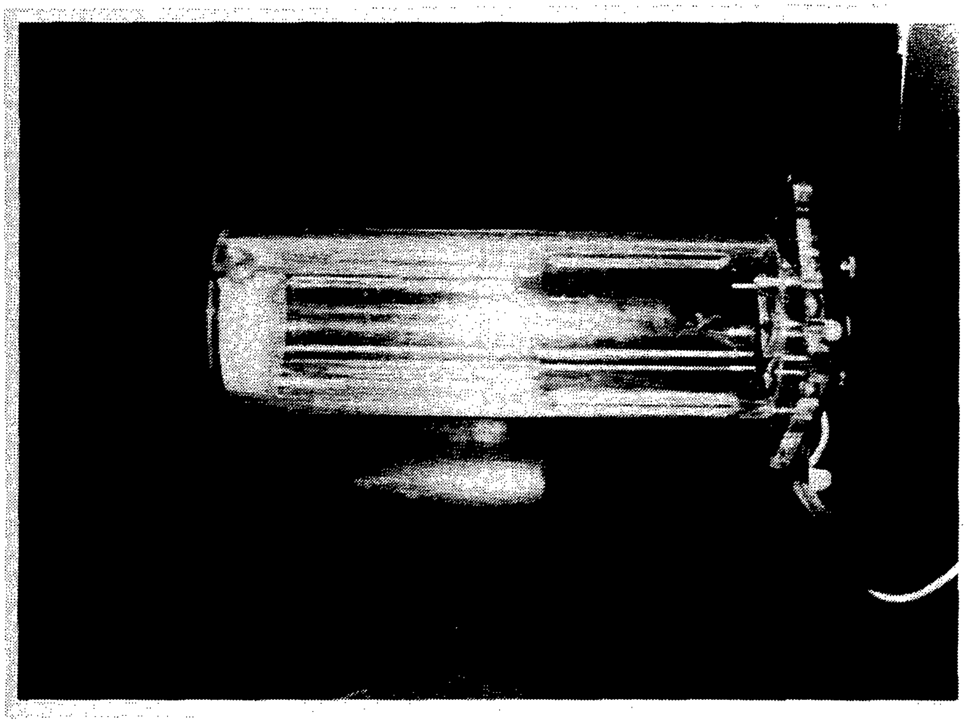


Figure 3a. Rat Restraint (photo)

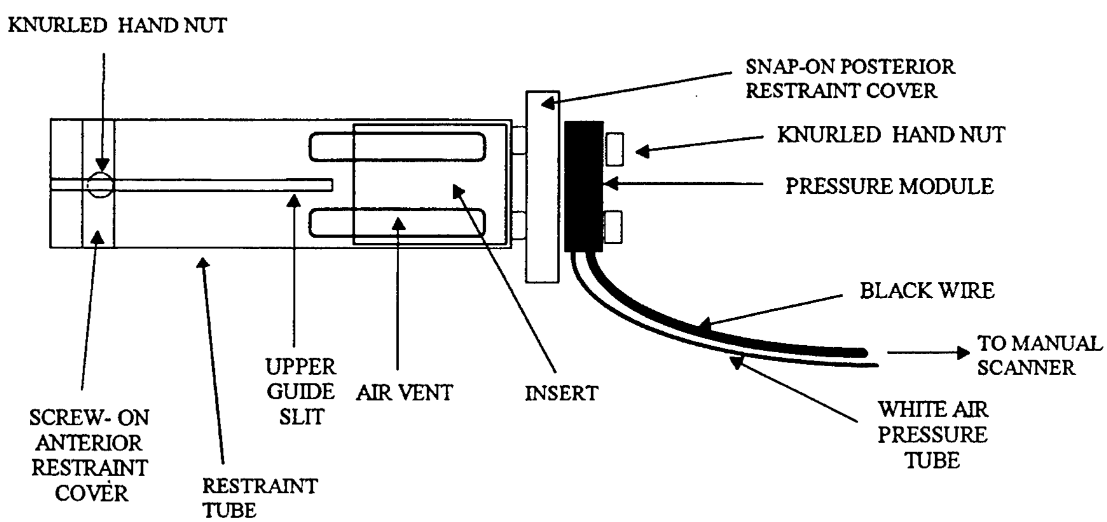


Figure 3b. Rat Restraint (diagram)

Temperature Equilibration

Conscious rats were placed in tubular restraints and housed within a clear, plexiglas ambient temperature chamber developed in-house (see photograph in Figure 4a and diagram in Figure 4b.). The rats were allowed 30 minutes to equilibrate prior to initiation of data acquisition. The outer dimensions of the chamber were 24 in. wide, 27 in. tall, and 16 in. deep. It contained four ventilated trays with a carrying capacity of three rats per tray. Each tray rested on top of a waste collection tray and was supplied at both ends with a continuous flow of outside air warmed to 29-30 °C by two thermostatically regulated heater fans. Air exited through gaps in the front of the box, between two sliding doors. The chamber was also used to dry and store empty restraint tubes and accessories after cleaning. In Figure 4, (third upper chamber) the rear of the restraint tube with attached pressure module faces towards the front of the chamber with the wire and air pressure tubing being threaded behind the doors and out one side. A cyclindrical radiotelemetric receiver sits parallel to and against the restraint tube. This was the arrangement used to collect tailcuff and implant data at the same time. It allowed adjustments to be made whenever an animal's tail began to slip out of the cuff. It also provided an easy means for removing the restraints for animal replacement and for removing equipment for individual chamber cleaning.

Radiotelemetric Monitoring

Equipment

Data Sciences Software (Version 2.22, 1994), a computer board Model DQ1188, a BCM 100 consolidation matrix module with a voltage regulating power plug, Model RA1010 flat receivers, Model RLA3000 cylindrical receivers, Model TA11PA-C40 radiotelemetric implants, and a Model C11PR ambient pressure monitor were purchased from Data Sciences, St. Paul, MN. The Data Sciences card was installed in a Zenith 386 computer containing 8 megabytes of RAM, an 80 megabyte hard drive, and a math coprocessor. The computer was loaded with OS/2 (Version 2.00). Included with the computer was a color monitor and a mouse. The computer was connected via the card to the BCM 100 consolidation matrix and the latter was connected to receivers. An overview of the system is shown in the photograph in Figure 5a and the diagram in Figure 5b.

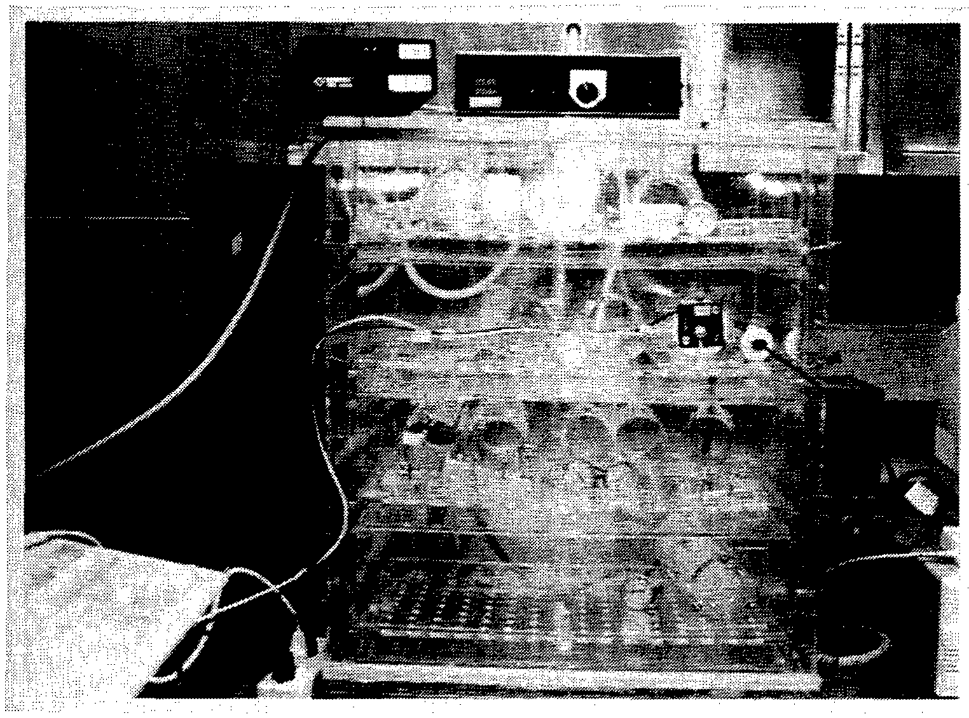


Figure 4a. Ambient Chamber (photo)

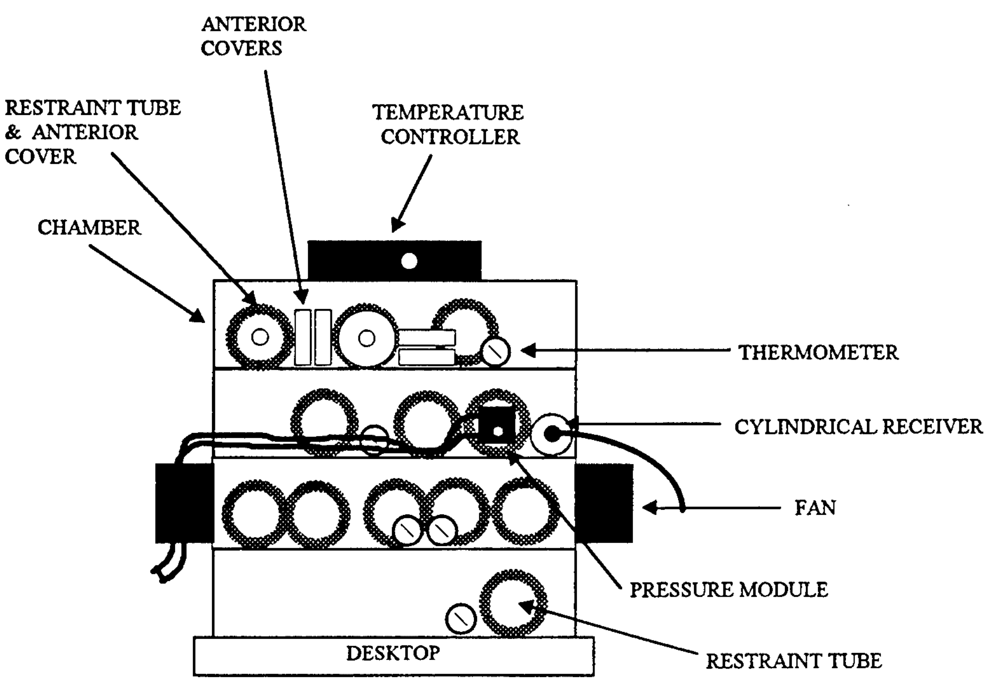


Figure 4b. Ambient Chamber (diagram)

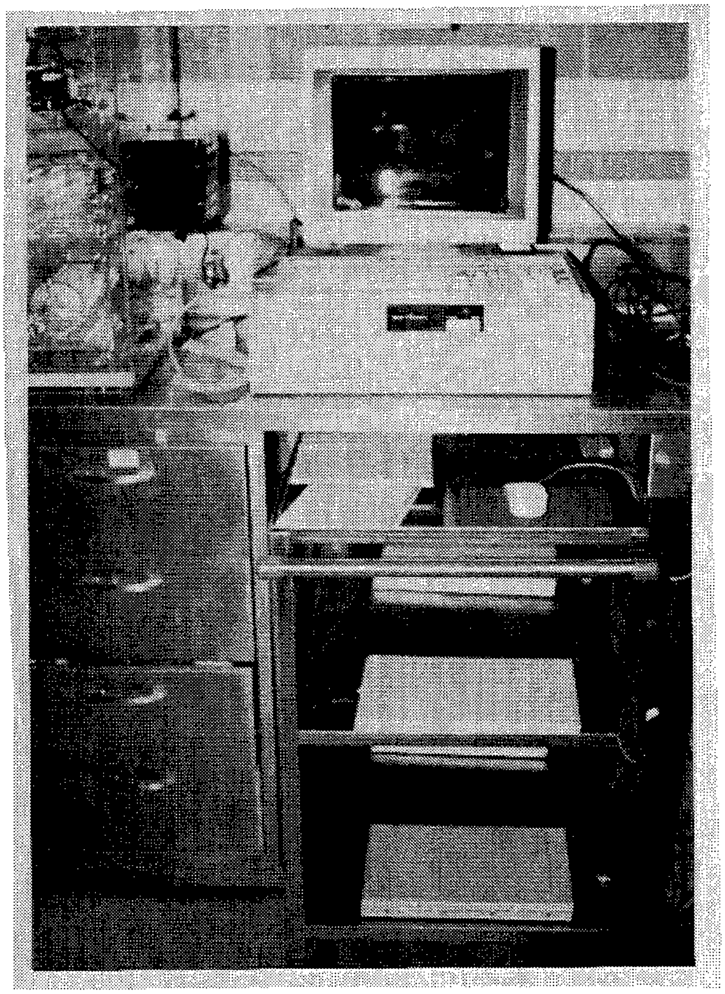


Figure 5a. Radiotelemeter Hardware
(photo)

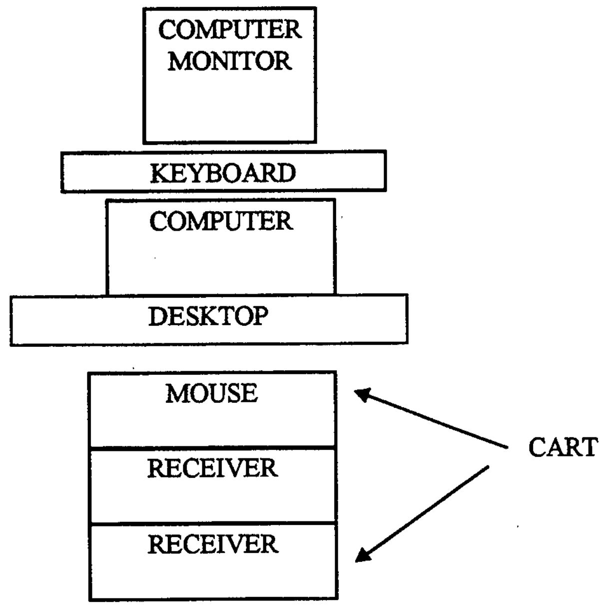


Figure 5b. Radiotelemeter Hardware (diagram)

The photograph in Figure 6a and the diagram in Figure 6b illustrate the consolidation matrix with the pressure monitor resting on top. To the side of the consolidation matrix is one of the cylindrical radiotelemetric receivers used to monitor blood pressure and heart rate within the ambient chamber. When the animals were monitored outside of the tubular restraints, their cages were placed on top of one of the flat receivers shown on the cart in Figure 5a. An implantable transmitter is shown in the photograph in Figure 7a and the diagram in Figure 7b.

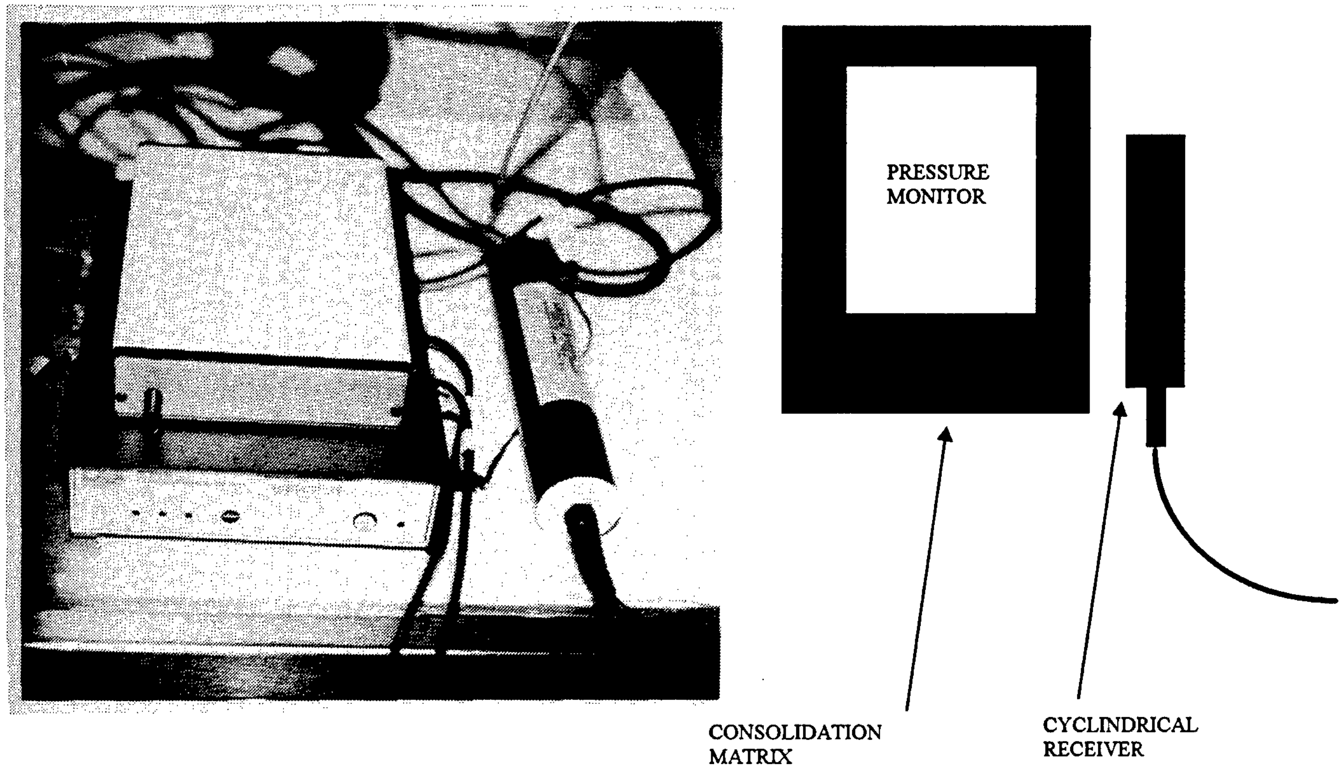


Figure 6a.(photo) and 6b.(diagram) Pressure Monitor, Consolidation Matrix, and Cylindrical Receiver

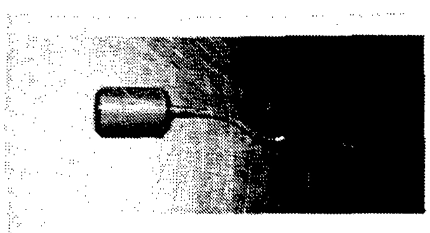


Figure 7a. Transmitter (photo)

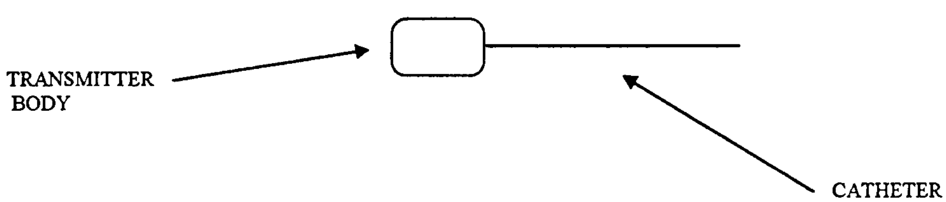


Figure 7b. Transmitter (diagram)

Transmitter Calibration

Transmitters were calibrated by the manufacturer at the factory. The transmitters were turned on with a strong magnet, checked for an audible signal using an AM radio tuned to a low frequency (500 kHz), and allowed to sit one day to equilibrate before determining offset values. The calibration values were typed into the "Configuration" program and the offsets checked in the "Data Display" window of the "Acquisition" program. Offset values did not deviate more than the recommended amount (0 + 3 mmHg). When removed from the animals, the transmitters were checked again for electronic drift. Offset values did not deviate more than the recommended amount (0 + 5 mmHg). Transmitters were recalibrated using the formula (cal2 - cal1)/(100) (offset) = adjustment value. The adjustment value was added to the current calibration values if it was positive or subtracted if it was negative.

Surgical Procedures

Rats were anesthetized by intraperitoneal injection with pentobarbital (50 mg/kg body weight). The abdomen was opened, the transmitter catheter tip was inserted into the abdominal aorta just above the lower bifurcation into the femoral arteries and threaded into the aorta just beneath the renal artery junction. The catheter was glued into place with a cellulose patch and surgical glue. The transmitter body was looped around so it was lying next to the catheter tip. This procedure minimized pressure head differences between the tip and the body regardless of animal orientation. Catheter insertion was verified using an AM radio. If insertion was successful, the monotone associated with the transmitter would change to a pulsating sound. The transmitter body was then sewn into the wall of the peritoneal cavity. The wound was sewn shut, skin stapled together and the animals were allowed to recover for several days prior to use. After removal from animals, catheters were soaked in saline for 48 hours to facilitate debris removal from the tip and the tips were regelled as needed. Catheters were cold sterilized by soaking them for 12 hours in cidex 7® (600 mL cidex, 17 mL activator) prior to surgery and rinsed one-half hour before use. A total of eight implanted rats were used throughout this study. Of these, five were used in comparing tailcuff to telemetric readings.

Electronic Noise

Transmitters were susceptible to outside electronic interference and to each other. Therefore, care was taken to insure "cross talk" did not occur between transmitters and that transmitters and receivers were kept away from equipment such as computer printers. See Figures 15-19 in the Appendix for examples of radiotelemetric wave tracings.

Data Acquisition

Data acquired included blood pressure (systole, mean, and diastole) and heart rate. Mean blood pressure was calculated from the highest value in the pulse wave generated during tailcuff monitoring. During radiotelemetric data acquisition, it was calculated as a weighted average (minus outliers) from a series of 500 data points collected during a one- second period. Diastolic pressure was not directly measureable by Tailcuff and was calculated indirectly from systolic and mean blood pressures using the formula DIA = (3MBP - SYS)/2.

Prior to implantation, rats were subjected for five consecutive days to two daily series of triplicate tailcuff analyses to acquire baseline readings from nonimplanted animals. Data acquired included blood pressure (systole, mean, and diastole) and heart rate. After implantation, telemetric readings of blood pressure and heart rate were acquired from five animals for 30 minutes within their cages. Ten second scans were recorded every five minutes. The animals (in restraining tubes) were placed within the ambient chamber and allowed to equilibrate for 30 minutes, during which time additional telemetric readings were taken. Finally, two sets of tailcuff readings were recorded from the animals (approximately 30 minutes), during which time a third set of telemetric readings was acquired. Data acquisition was performed in this manner for five consecutive days. Tailcuff readings, taken before and after implantation, were used to evaluate the effects of implantation on blood pressure and heart rate. Conversely, implant readings taken during tailcuff monitoring were used to evaluate the effects of tailcuff inflation on blood pressure and heart rate. Tailcuff and implant readings taken within the same time frames were used to correlate blood pressure and heart rate measurements obtained by the two methodologies. Effects of implantation on body weight gain and terminal liver-to-body weight ratios were also determined. Gross and histopathologic evaluations of livers from implanted animals and one control were also performed to determine if any damage had occurred from the close proximity of the transmitter body to the liver.

Data Analysis

Tailcuff data was inspected and recalculated as needed (see Figures 20 and 21 in the Appendix). Tailcuff and implant data were consolidated into Microsoft Excel spreadsheets and uploaded into RS1 for subsequent in-house analyses by BMDP and SAS statistical programs. Blood pressure and heart rate readings acquired for 5 consecutive days from 5 implanted animals were pooled for statistical evaluation. A 5-day total of 113 tailcuff readings were used for tailcuff data acquired prior to radiotelemetric implanation (Basecuff). A 5-day total of 108 tailcuff readings were utilized from the same animals after implantation and recovery from surgery. A 5-day total of radiotelemetric readings obtained from 10-second scans acquired every 5 minutes were obtained from these same animals. Implant readings were separated into three groups according to when they obtained. The first group was acquired from the animals during a 30-minute period when they were allowed to roam freely within their cages [Implants (1)]. A total of 153 readings were pooled in this group. At this point, data acquisition had been interrupted, animals placed into restraining tubes, connected to tail cuff monitors, and housed within an ambient temperature chamber. Data acquisition was resumed during this 30-minute temperature equilibration period [Implants (2)]. A total of 150 readings were used from this group. The third group of implant readings [Implants (3)] were acquired immediately following the temperature equilibration period during the time period tailcuff readings (Expcuff) were being obtained. A total of 67 readings were used from this group. The "Expcuff" readings and "Implants (3)" readings from the 5 animals were not collected simultaneously. They were matched as closely in time as possible and averaged into 23 matched data sets to correlate blood pressure and heart rate readings obtained from Tailcuff and Implants.

SECTION 3

RESULTS

Tables

Body weight comparisons were made between nonimplanted animals (Controls) and implanted animals (Implants) 48-49 days postimplantation (Table 1). Liver-to-body weight ratios were established for controls and Implants 52-54 days postimplantation. Independent, two-sample t-tests indicated no significant differences between body weights or liver/body weight ratios of control and implanted animals. Necropsy results (not shown) demonstrated slight liver discoloration at points where adhesion tissue had attached the liver to a partially or fully

ratios of control and implanted animals. Necropsy results (not shown) demonstrated slight liver discoloration at points where adhesion tissue had attached the liver to a partially or fully encapsulated transmitter body. Otherwise, no gross pathological differences were noted between controls and implanted animals. Transmitters removed from the animals were checked repeatedly over a period of time and found to be within 5 mmHg from zero, which was within acceptable limits.

TABLE 1. COMPARISON OF WEIGHT GAIN AND LIVER/BODY WEIGHT RATIOS IN CONTROLS VERSUS IMPLANTED ANIMALS

	Pre-weight	Post-weight	Liver/Body Weights
Controls	501 ± 09	592 ± 11	0.0495 ± 0.0028
Implants	467 ± 36	537 ± 43	0.0475 ± 0.0017

Data are the means $\pm$ SEM.
No significant differences between controls and Implants.
N= number of animals used.
N = 8 for data in columns 2 and 3.
N = 5 for data in column 4.

Three sets of data were compared from five rats (Table 2). These data were blood pressure and heart rates acquired from tailcuff measurements prior to implantation (Basecuff), after implantation (Expcuff), and implant readings acquired during the same time frames as Expcuff readings (Implants). The data obtained include systolic blood pressure (SYS), mean blood pressure (MBP), diastolic blood pressure (DIA), and heart rate (HR). Blood pressure and heart rates measured by tailcuff were similar to those cited in the literature (Bivin, Crawford, and Brewer, 1979). A two-sample, multivariate, independent t-test indicated no significant differences in the means of any of the blood pressure parameters or heart rate between Basecuff and Expcuff. In contrast, significant differences between Expcuff and Implants were noted in the means of the three blood pressure parameters and heart rate at $p < 0.01$. The implant blood pressure readings were about 20-30 mmHg higher than those obtained by Tailcuff. Heart rate readings for Implants were 14-16 beats/min faster than those derived by Tailcuff. Coefficients of variation for tailcuff blood pressure readings (not shown) were approximately 1.75-3 times larger than those obtained from Implants, the latter being remarkably consistent between systolic, mean, and diastolic blood pressures. Tailcuff blood pressure variability was highest in diastolic readings, lower in mean blood pressure, and lowest in systolic readings.

TABLE 2. COMPARISON OF IMPLANT READINGS TO TAILCUFF READINGS TAKEN BEFORE AND AFTER IMPLANTATION

	Basecuff	Expcuff	Implants (3)
SYS	119 $\pm$ 1.5	118 $\pm$ 1.5	141 $\pm$ 1.4 [a]
MBP	90 $\pm$ 1.6	94 $\pm$ 1.6	119 $\pm$ 1.2 [a]
DIA	75 $\pm$ 2.0	81 $\pm$ 1.9	100 $\pm$ 1.0 [a]
HR	339 $\pm$ 3.2	341 $\pm$ 3.0	355 $\pm$ 4.7 [a]

Systole (SYS), mean blood pressure (MBP) and Diastole (DIA) are reported as mmHg.
Heart Rate (HR) is reported as beats per minute.
Data are the means + SEM.
Basecuff is tailcuff data taken before implantation.
Expcuff is tailcuff data taken after implantation.
Implants (3) is radiotelemetric data taken during the same time frame as Expcuff data.
No significant differences between Basecuff and Expcuff.
[a] Significantly different from Expcuff at $p < 0.01$.
N= number of readings obtained for a pooled set of 5 animals.
N = 113, 108, and 67 for Basecuff, Expcuff, and Implants (3), respectively.

Three sets of data were collected daily from the five implanted rats (Table 3). The first set are data obtained for 30 minutes during which time the animals were allowed to roam freely within their cages (Implants (1)). The second set are data obtained when the animals were placed into restraining tubes, placed into an ambient temperature box (29-30 ^{o}C), fitted with tailcuff monitors and allowed to acclimatize for 30 minutes (Implants (2)). The third set of data were obtained after the animals acclimatized for 30 minutes and were subjected to tailcuff monitoring (Implants (3)). The readings obtained include systolic blood pressure (SYS), mean blood pressure (MBP), diastolic blood pressure (DIA), and heart rate (HR). No significant differences were noted between Implants (1) and Implants (2) blood pressure readings as determined by two-sample, multivariate, independent t-tests. However, significantly lower differences were noted between Implants (1) or Implants (2) versus During tailcuff blood pressure readings at $p < 0.01$ for SYS and MBP and $p < 0.05$ for DIA. Significantly higher differences in heart rate readings were noted in Implants (1) versus Implants (2) and Implants (1) versus Implants (3) at $p < 0.01$ and 0.05 respectively. Implants (3) was higher than Implants (2) though the differences were not significant.

TABLE 3. IMPLANT READINGS BEFORE ANIMAL RESTRAINT, AFTER RESTRAINT, AND DURING TAILCUFF MONITORING

	Implants (1)	Implants (2)	Implants (3)
SYS	134 $\pm$0.5	135 $\pm$1.0	141 $\pm$1.4[a]
MBP	113 $\pm$0.5	114 $\pm$0.8	119 $\pm$1.2[a]
DIA	95 $\pm$0.5	97 $\pm$0.7	100 $\pm$1.0[b]
HR	384 $\pm$3.4	345 $\pm$3.0[c]	355 $\pm$4.7[d]

Systole (SYS), mean blood pressure (MBP) and Diastole (DIA) are reported as mm Hg.
Heart Rate (HR) is reported as beats per minute.
Data are the means + SEM.
Implants (1) data were taken before animal restraint, beginning 60 minutes prior to tailcuff readings.
Implants (2) data were taken during animal restraint and equilibration beginning 30 minutes prior to tailcuff readings.
Implants (3) data were taken as Expcuff tailcuff readings were being acquired.
No significant differences between blood pressure readings for Implants (1) and Implants (2).
a Significantly different from Implants (2) at $p < 0.01$.
b Significantly different from Implants (2) at $p < 0.05$.
c Significantly different from Implants (1) at $p < 0.01$.
d Significantly different from Implants (1) at $p < 0.05$.
N= number of readings obtained for a pooled set of 5 animals.
N = 153, 150, and 67 for Implants (1), Implants (2) and Implants (3) respectively.

Correlation coefficients were determined between systolic, mean, and diastolic blood pressures and heart rate to measure how internally consistent these readings were from data acquired by Tailcuff and Implants. Correlation coefficients included systolic versus mean blood pressures (SYS/MBP), systolic versus diastolic pressures (SYS/DIA), mean versus diastolic pressures (MBP/DIA), systolic pressure versus heart rate (SYS/HR), mean pressure versus heart rate (MBP/HR), and diastolic pressure versus heart rate (DIA/HR). Positive correlations for all three blood pressure parameters versus each other were significant at $p < 0.0001$ (Table 4) as determined by the Standard Pearson Correlation Test (Rosner, 1990). Tailcuff blood pressure correlations were considerably smaller than those obtained for Implants. Correlations in tailcuff measurements for all three blood pressure parameters versus heart rate were poor and insignificant or were inversely correlated. In contrast, these same measurements for Implants were all positively correlated and significant at $p < 0.0001$. Even so, they were considerably less correlated than any of the three blood pressure parameters versus each other.

TABLE 4. INTERNAL CORRELATIONS OF BLOOD PRESSURE AND HEART RATE PARAMETERS

	Basecuff	Expcuff	Implants (3)
SYS/MBP	0.65[a]	0.72[a]	0.98[a]
SYS/DIA	0.40[a]	0.50[a]	0.89[a]
MBP/DIA	0.96[a]	0.96[a]	0.93[a]
SYS/HR	-0.43[a]	-0.13	0.47[a]
MBP/HR	-0.21[b]	0.10	0.57[a]
DIA/HR	-0.09	0.18	0.62[a]

Systole (SYS), mean blood pressure (MBP) and Diastole (DIA) are reported as mmHg.
Heart Rate (HR) is reported as beats per minute.
Basecuff is tailcuff data taken before implantation.
Expcuff is tailcuff data taken after implantation.
Implants (3) is radiotelemetric data taken during the same time frame as Expcuff data.
a Significant at $p < 0.0001$
b Significant at $p < 0.02$
N= number of pooled readings obtained from 5 animals over 5 consecutive days.
N = 113, 108, and 67 for Basecuff, Expcuff and Implants (3), respectively.

Correlations between readings obtained by Tailcuff (n = 108) and Implants (n = 67) within the same time frames were calculated to measure the degree of consistency between the two techniques. Data sets acquired within five-minute intervals were averaged to generate 23 pairs of matching data (Table 5). Data for the three blood pressure parameters and heart rate were compared using the Standard Pearson Correlation Test (Rosner, 1990). Correlations for systolic pressure, mean pressure, diastolic pressure, and heart rate were 0.53, 0.40, 0.24, and 0.62, respectively. Correlations for systolic pressure were significant at $p < 0.01$. Correlations for mean pressure and diastolic pressure were not significant. Correlations for heart rate were significant at $p < 0.002$.

TABLE 5. CORRELATIONS OF IMPLANT (3) READINGS TO EXPCUFF READINGS

	r
SYS	0.53[a]
MBP	0.40
DIA	0.24
HR	0.62[b]

Systole (SYS), mean blood pressure (MBP) and Diastole (DIA) are reported as mmHg.
Heart Rate (HR) is reported as beats per minute.
Expcuff is tailcuff data taken after implantation.
Implants (3) is radiotelemetric data taken during the same time frame as Expcuff data.
a Significant at $p < 0.01$.
b Significant at $p < 0.002$.
N = 23 for averaged data sets of Expcuff and Implants (3) closely matched in time.

SECTION 4

DISCUSSION

Correlations of tailcuff blood pressure readings (systole, mean blood pressure, and diastole) to each other were highly significant ($p < 0.0001$). However, correlations of systole to mean blood pressure and diastole were considerably smaller than parallel correlations obtained by implantation. Although mean blood pressure versus diastole was equivalent in Tailcuff and Implants, this was probably due to direct calculation of diastole from mean blood pressure and systole in the tailcuff procedure. The poorer correlation of systole and diastole in the tailcuff procedure compared to diastole and mean blood pressure may reflect the fact that mean blood pressure was more heavily weighted than systole in diastole determination (diastolic = [3 x mean - systolic]/2). Interestingly, the tailcuff correlations improved during the second week of monitoring (after implantation), supporting the hypotheses that additional training improves tailcuff data. It also demonstrates that implantation has no adverse effect on blood pressure readings taken by tailcuff.

One reason for the smaller tailcuff correlations may be the requirement for occasional manual redrawing of tailcuff curves, which introduces subjectivity into the readings. Another possibility may be thermoregulation in the tail. Some rats were unable to generate good pulse readings, even after 30 minutes acclimatization at 30 °C. *Note: After the study was completed, some unresponsive rats were briefly heated to about 35 °C and allowed to cool down in the ambient chamber. Subsequently, they were able to give consistently good pulse readings. It was decided that localized heating of the tail itself within the ambient chamber may be useful (see also Bunag and Butterfield,1982). Preliminary findings suggest that covering the tail with styrofoam to induce localized heating may allow reductions in ambient temperature and acclimatization times while enhancing pulse readings from unresponsive rats. Whether or not this would increase tailcuff correlation coefficients has not been determined.*

The modest increase in blood pressure during tailcuff inflation (as measured by Implants) may be the result of either pyschological stress on conscious animals as a result of the localized

pressure on their tails, or because constriction of the artery increases pressure in the abdominal aorta where the implant is taking readings. These alternative hypotheses could be tested by monitoring blood pressure from anesthetized animals, thereby eliminating any blood pressure changes due to pyschological stress.

The tailcuff data is in good agreement with other studies described in the literature (Bidani et al., 1993; Bunag and Butterfield, 1982), including one study involving Implants (Guiol et al., 1992). Higher blood pressure ranges for tailcuff studies in Sprague-Dawley rats have also been described (Spanos et al., 1991).

However, absolute blood pressure and heart rate measurements are less critical than the ability of the procedure(s) to detect changes in data from baseline values in response to exposure to vasoactive substances. For this purpose, it appears that the implant procedure has several advantages over Tailcuff: 1) It is far more convenient and humane to monitor the animals because they require no restraints, and hence, no training. More daily readings can be obtained because the requirement for "breaks" between restraining periods is unnecessary. Unsupervised readings can be taken at night when the animals are feeding, which is highly advantageous in situations where the test compound is contained in the feed. 2) Internal correlations of systole, mean blood pressure, diastole, and heart rate are generally higher in Implants than they are in the tailcuff procedure. 3) Readings can be taken immediately and continuously with the implant method, whereas with the tailcuff procedure, the animals have to be heated for at least 30 minutes up to 30 ^{o}C to get adequate readings. Even with this procedure, some of the animals fail to generate adequate pulses. 4) Implantation did not appear to have an effect on tailcuff blood pressure readings as determined by comparing differences in tailcuff readings between pre-implanted and post-implanted animals. In contrast, tailcuff monitoring did slightly elevate implant readings of blood pressure and heart rate. The major disadvantages associated with transmitters is their capacity for electronic "drift" and interference by outside electromagnetic fields. Electronic drift may be minimized by making certain that transmitters and pressure monitors are properly calibrated and studies are kept as short as possible between calibration checks. Interference by stray electromagnetic fields must be dealt with empirically; i.e., avoid placing the receivers in a room or area that generates interference.

SECTION 5

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SECTION 6

APPENDIX A

Examples of Radiotelemetric Readings

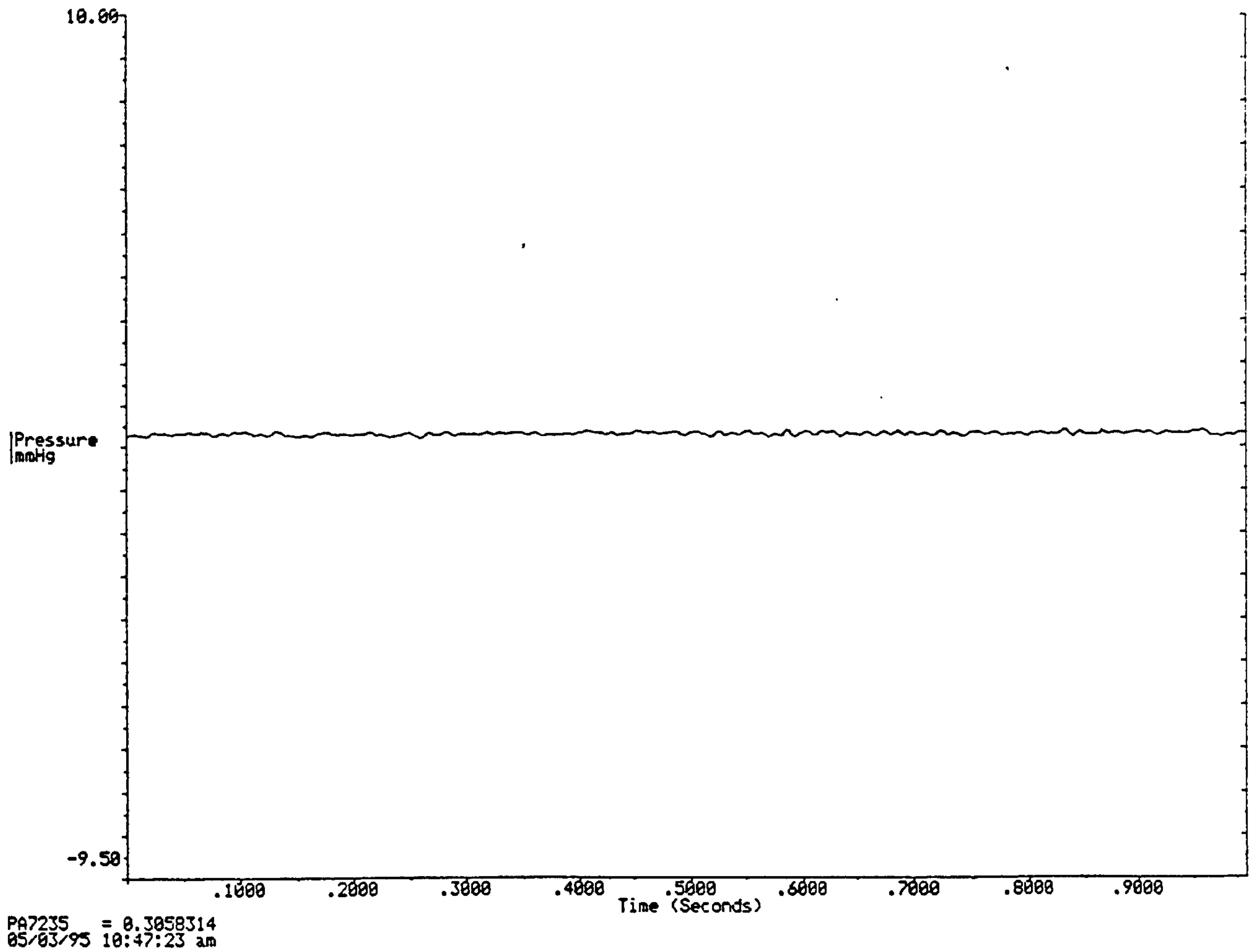

Figure 8. Example of a baseline reading with little noise interference

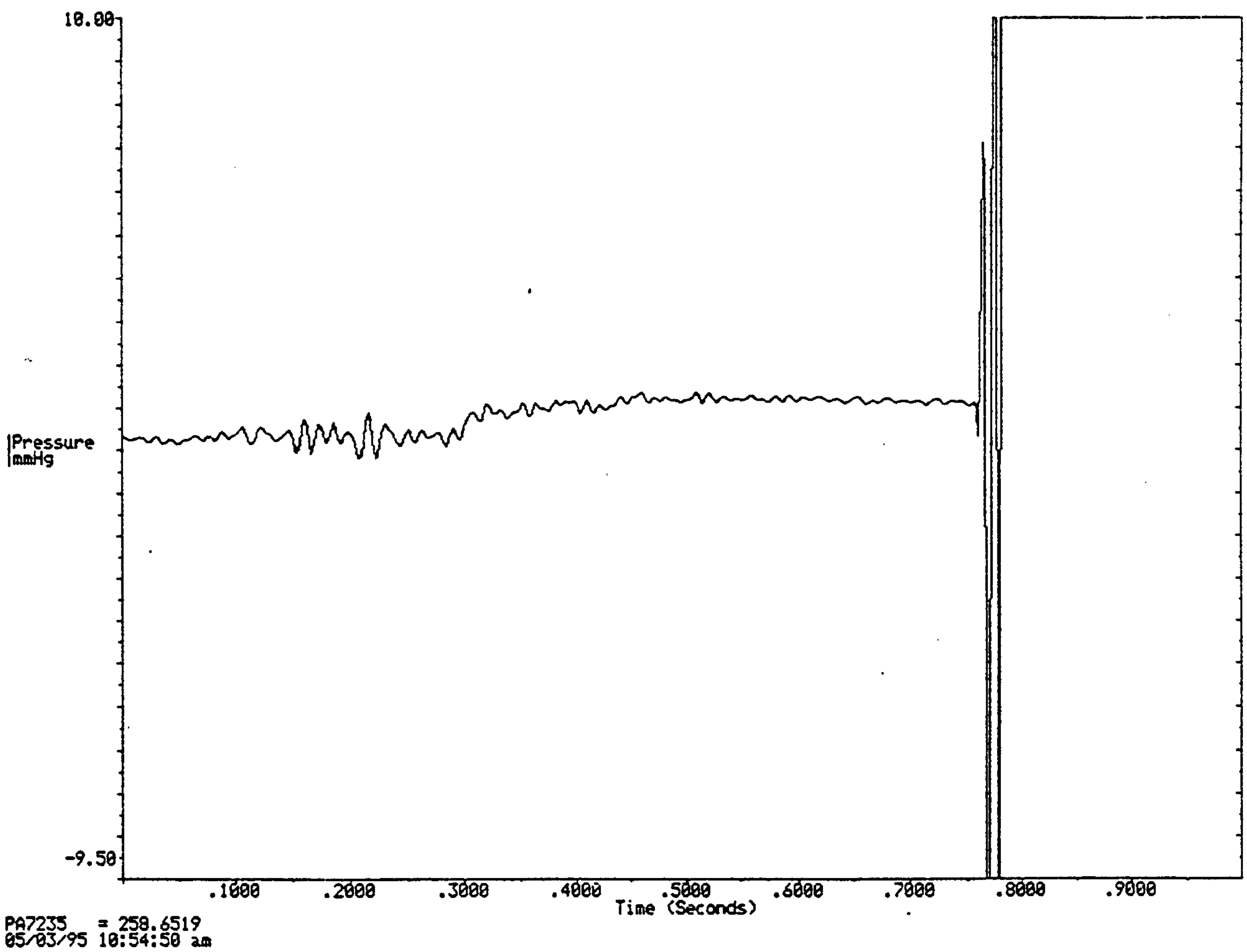

Figure 9. Example of electronic noise "cross talk" between closely spaced transmitters (1 second scan)

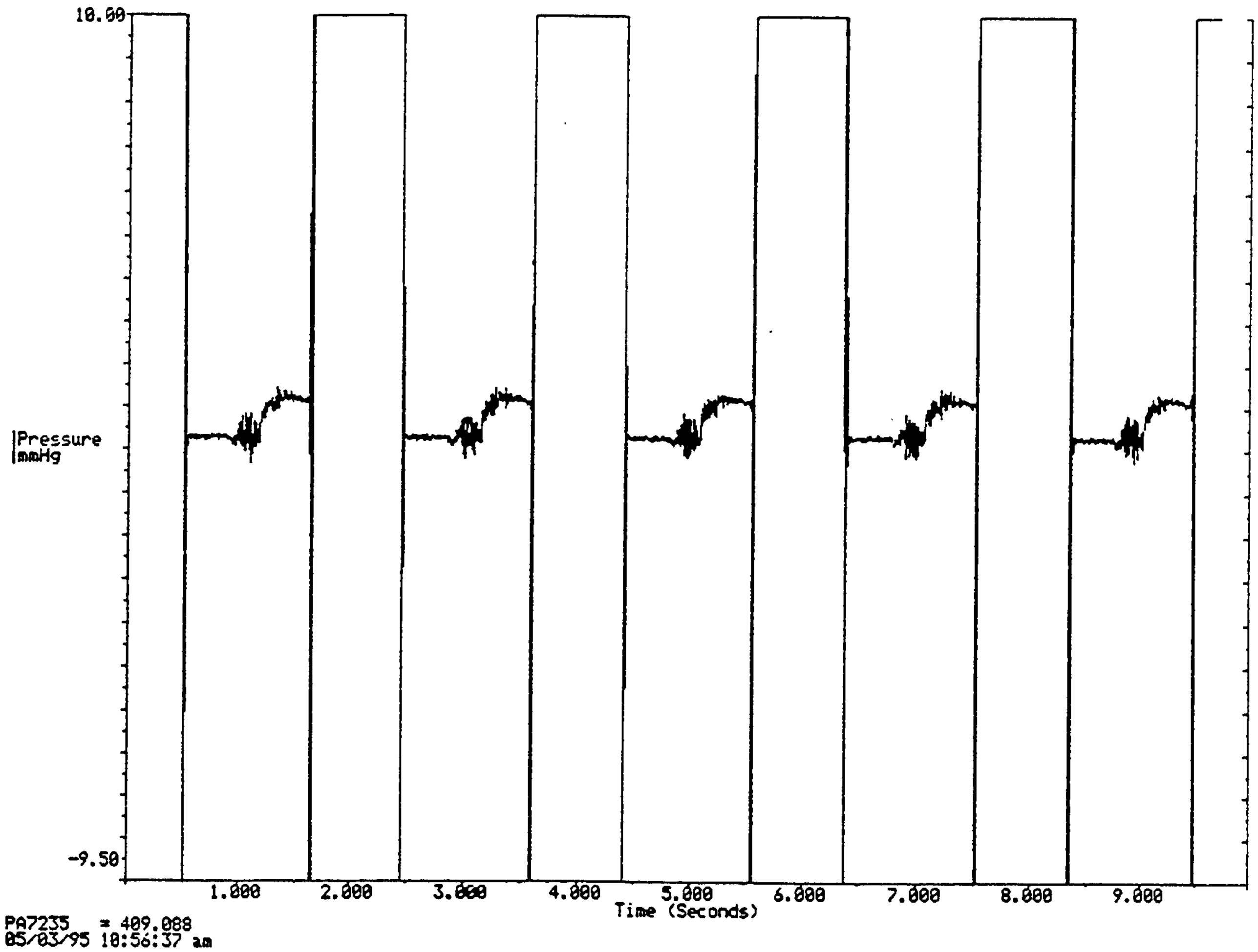

Figure 10. Example of electronic noise "cross talk" between closely spaced transmitters (10 second scan)

Pulse Wave Generated by a Radiotelemetric Implant

Upper portion of wave is systolic pressure, lower end of wave is diastolic pressure. Data from the pulse wave is averaged every 5 minutes over a 10 second interval.

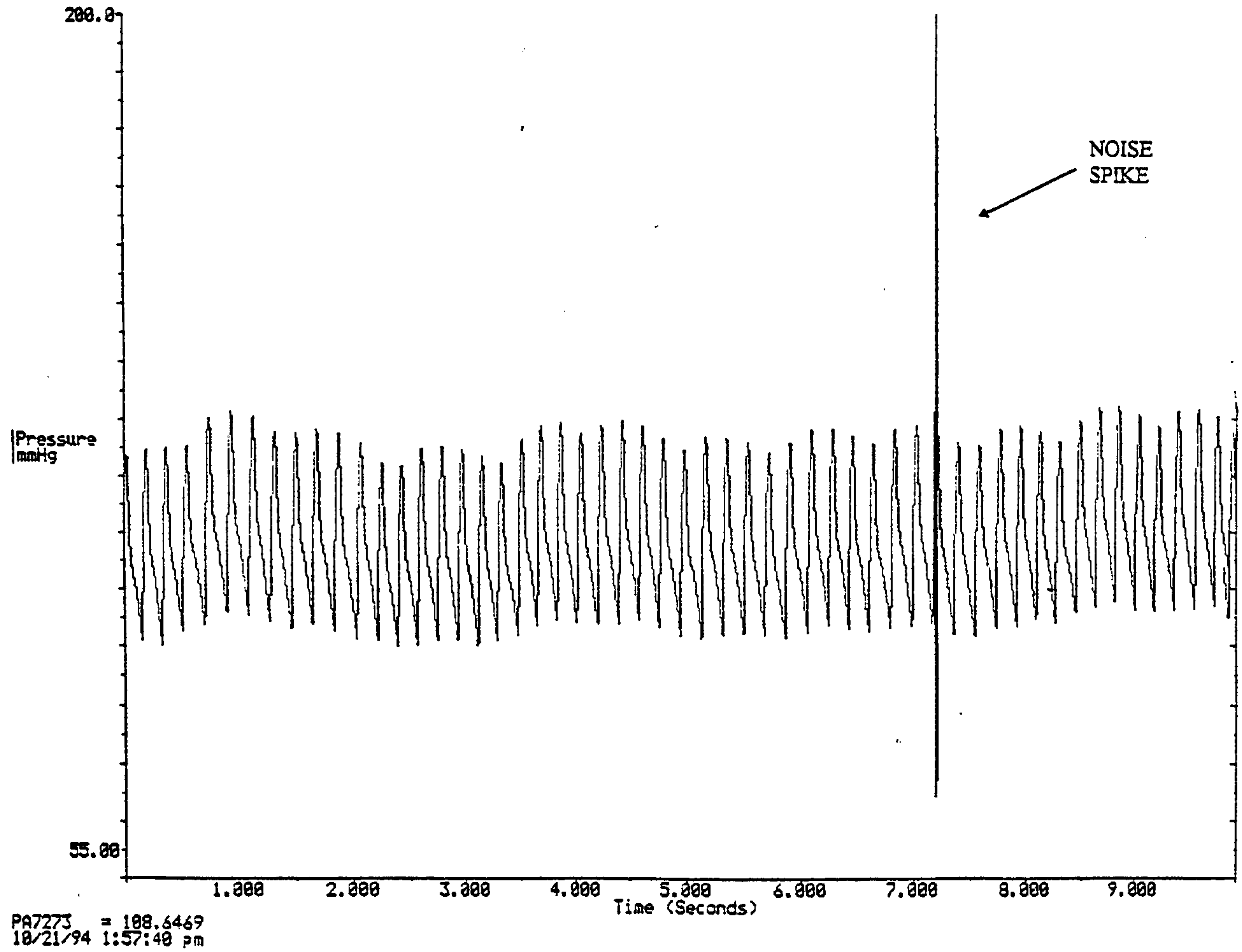

Figure 11. Example of a good blood pressure reading with little noise interference

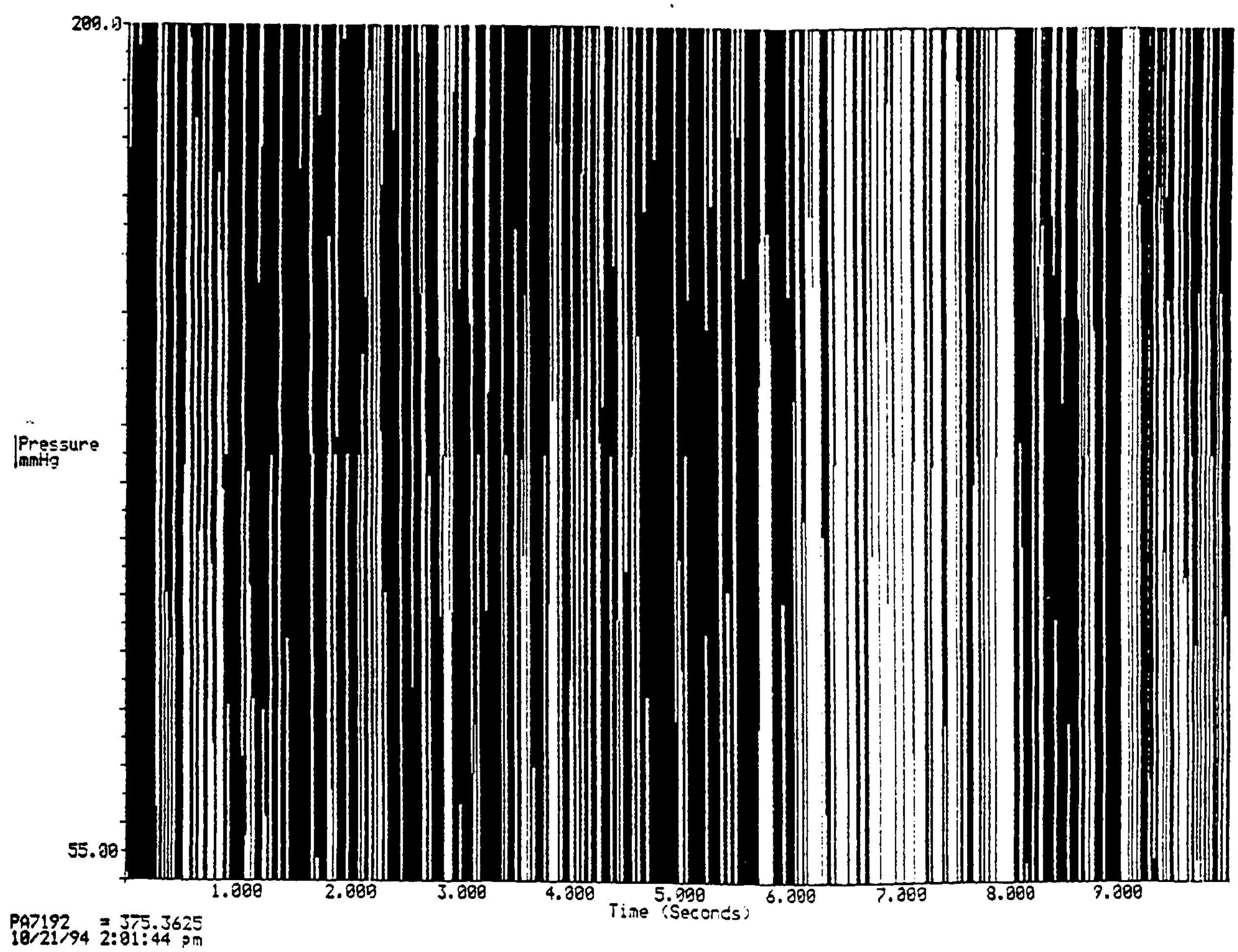

Figure 12. Example of a bad blood pressure reading with noise interference from a printer

Examples of Tailcuff Pulse Reading

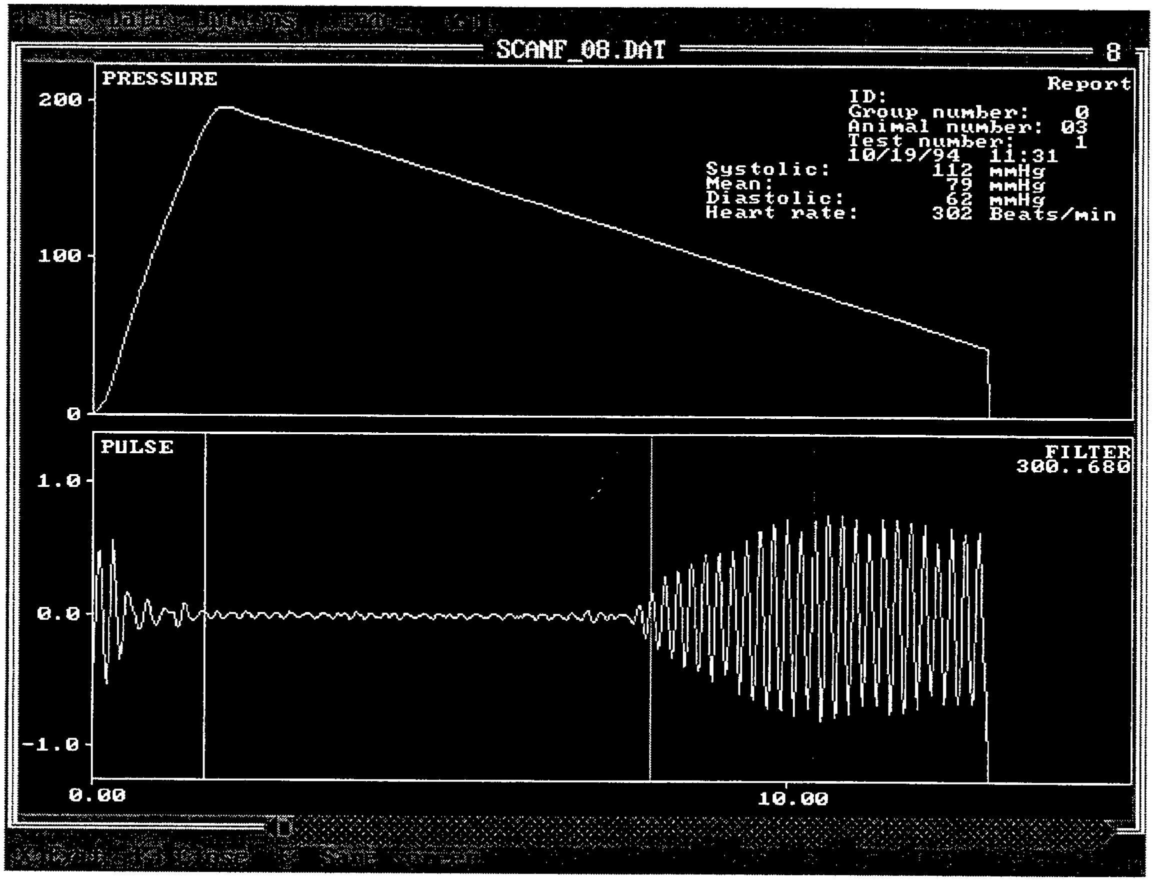

Figure 13. Example of a Good Pulse Reading

Figure 13 is an example of a good blood pressure reading. The upper chart illustrates the rapid increase in cuff pressure (mm Hg) over time (seconds) during inflation followed by a linear decrease and then a sudden drop at the end as the residual pressure is completely released.

The computer-generated readings are shown in the upper right hand corner of the pressure chart. The animal number is the module number. The test number indicates the number of times that module has been inflated during that cycle. Each completed test is given a file name. In this particular example the name is, "SCANF_08.DAT" (upper middle area of the pressure chart). "SCANF" is the default name given to all new files.

The lower chart is the pulse chart which is recorded over time in $\pm$ millivolts. This is the chart which is critical to understand in order to determine if the reading is good, requires manual manipulation, or should not be used at all. Unfortunately, there is a considerable degree of subjectivity involved in determining whether a reading should be adjusted and if so, how. Therefore, it is quite important that only one person is involved in doing these manipulations so that the readings will be adjusted in a consistent manner throughout any given set of

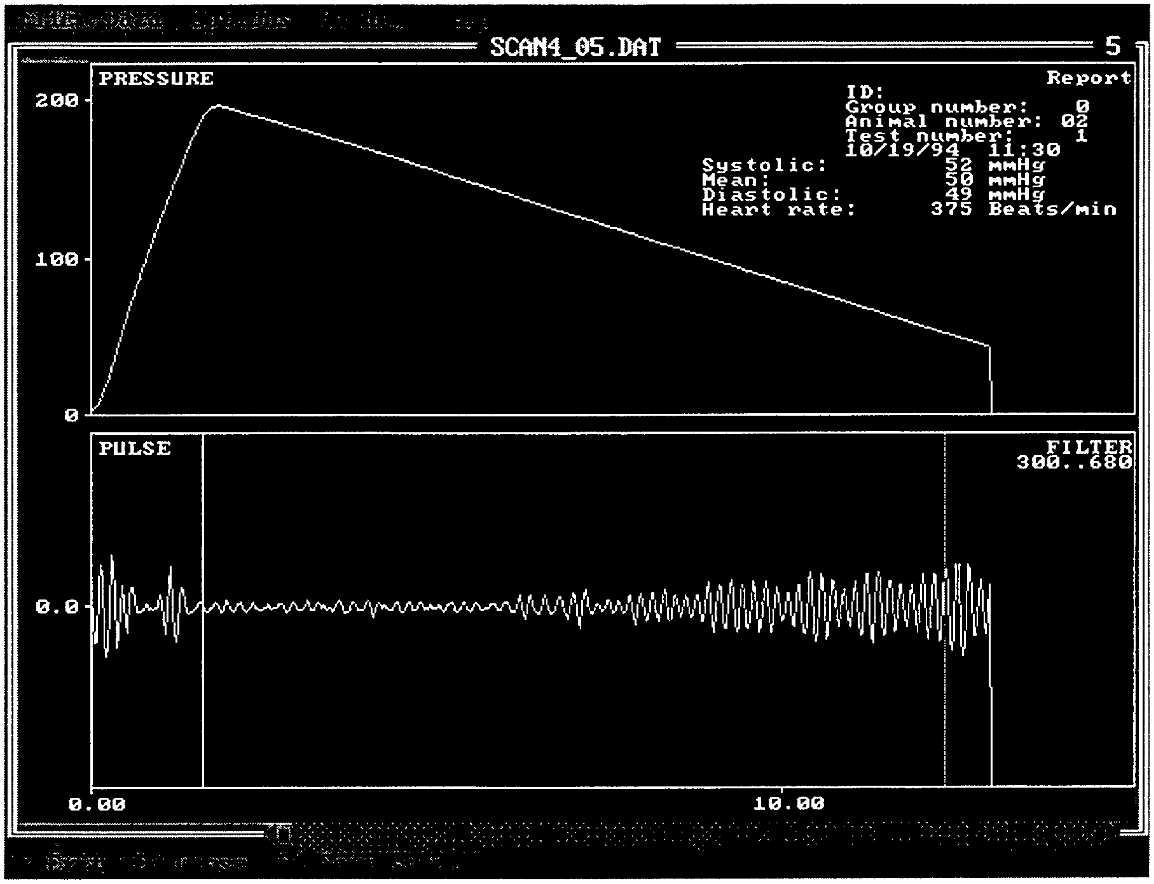

Figure 14. Example of a Poor Pulse Reading

The lower reading is adjusted by manipulating the green and red vertical demarcation lines with the computer left and right hand mouse buttons respectively. In the black & white illustration the "yellow" line is the more solid line fartherest to the left of the pulse chart. The dotted "green" line is next followed by the thinner dotted "red" line. The yellow line indicates when the computer begins to look for pulse signals. This corresponds to the inititation of cuff deflation (upper chart). The green line indicates the point the computer decides best represents the beginning of the true pulse pressure. The pressure at this point is calculated as the systolic pressure. The red line indicates the pulse wave with the highest amplitude in the pulse series. The computer registers this pressure as the mean blood pressure. Diastolic pressure is automatically calculated from the systolic and mean blood pressures. Note that between the yellow and green lines is a fairly quiet trace area. The filter helps to remove pulse artifacts due to breathing and gross animal movements. The pulse pattern in Figure 13 is well-defined and shaped like a football truncated on the right side. It rises quickly to a maximum amplitude then falls off in a symmetrical pattern. The truncation of the pattern occurs when the test is abruptly ended, which is a good indication of a successful test.

Compare this pulse tracing to the one in Figure 14. Note how close the green and red lines are on the tracing. Note the poor blood pressure readings in the upper right hand corner of the pressure chart. Also, note that there is no clearly defined symmetrical pulse pattern. Instead there is a continuously small and irregular pattern which rises and falls periodically, confusing the computer about where the systolic and mean blood pressure readings should be taken. This reading is irredeemable and should not be used.